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Endogenous resident c-Kit cardiac stem cells increase in mice with an exercise-induced, physiologically hypertrophied heart

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Abstract
Physical activity evokes well-known adaptations in the cardiovascular system. Although exercise training induces cardiac remodeling, whether multipotent stem cells play a functional role in the hypertrophic process remains unknown. To evaluate this possibility, C57BL/6 mice were subjected to swimming training aimed at achieving cardiac hypertrophy, which was morphologically and electrocardiographically characterized. Subsequently, c-Kit$^+$Lin$^-$ and Sca-1$^+$Lin$^-$ cardiac stem cells (CSCs) were quantified using flow cytometry while cardiac muscle-derived stromal cells (CMSCs, also known as cardiac-derived mesenchymal stem cells) were assessed using in vitro colony-forming unit fibroblast assay (CFU-F). Only the number of c-Kit$^+$Lin$^-$ cells increased in the hypertrophied heart. To investigate a possible extracardiac origin of these cells, a parabiotic eGFP transgenic/wild-type mouse model was used. The parabiotic pairs were subjected to swimming, and the wild-type heart in particular was tested for eGFP$^+$ stem cells. The results revealed a negligible number of extracardiac stem cells in the heart, allowing us to infer a cardiac origin for the increased amount of detected c-Kit$^+$ cells. In conclusion, the number of resident Sca-1$^+$Lin$^-$ cells and CMSCs was not changed, whereas the number of c-Kit$^+$Lin$^-$ cells was increased during physiological cardiac hypertrophy. These c-Kit$^+$Lin$^-$ CSCs may contribute to the physiological cardiac remodeling that result from exercise training.

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Introduction

Exercise training promotes a series of adjustments in the myocardium, with the main morphological adaptations attributed to hypertrophy — a stereotyped response of the heart (Abel and Doenst, 2011), which results in an increase in the cardiac chamber with a proportional change in wall thickness (McMullen and Jennings, 2007) and which constitutes a major mechanism of cardiac muscle adaptation against volume overload.

Cardiomyocyte hypertrophy is the dominant contributor to exercise-induced cardiac enlargement (Mann and Rosenzweig, 2012). Until recently in the history of medical science, it was believed that the heart was a static and post-mitotic organ, without regenerative capacity (Beltrami et al., 2003; Ellison, Waring, Vicinanza, and Torella, 2012; Chan, Shueh, Liu, and Hsieh, 2009); i.e., the number of cardiomyocytes in an individual would be established at birth (Kajstura et al., 2010; Barile, Messina, Giacomello, and Marbán, 2007). However, it is currently known that cardiomyocytes are renewed throughout life, albeit at very low rates. Compelling evidence suggests cell turnover and an intrinsic regenerative capacity in the mammalian heart (Barile et al., 2007).

Accordingly, concomitant with the cardiomyocyte hypertrophy, the number of heart cells also increases in response to exercise (Waring et al., 2014), while the physiologically hypertrophied heart maintains its normal cardiac structure. Although they are exceptionally complex cells with contractile fibrils organized into sarcomeres, cardiomyocytes, especially the small and immature cardiomyocytes, are capable of undergoing mitosis, albeit at very low rates (Bersell et al., 2009). However, this ability to divide is dependent on the presence of an inducing stimulus and is still quite limited in binucleated cardiomyocytes (Bersell et al., 2009). Therefore, in addition to the cardiomyocyte hypertrophy in the hearts of trained individuals, the cardiac cell number would also increase due to the division of pre-existing cardiomyocytes.

However, research in the last few years also showed that heart tissue homeostasis is maintained by the presence of cardiac stem cells (CSCs), which are organized into niches in the heart (Barile et al., 2007). These CSCs include c-Kit$^+$Lin$^-$, Sca-1$^+$Lin$^-$ and cardiac muscle-derived stromal cells (CMSCs, also known as cardiac-derived mesenchymal stem cells). The c-Kit$^+$Lin$^-$ and Sca-1$^+$Lin$^-$ cells can be distinguished via specific phenotypic markers and have clonogenic and self-renewal capacity, as well as the capability to differentiate into the three major cardiac lineages: myocytes, endothelial cells and smooth muscle cells (Gambini et al., 2011; Wang et al., 2014). CMSCs, which occupy the perivascular niche, have an important role in maintaining the integrity of matrix, stroma and vessels of the heart and also in contributing to parenchymal homeostasis, especially in injury and disease (Chong et al., 2011).

In cardiac hypertrophy associated with pathological processes, it has been shown that CSCs participate in the reparative processes (Ellison et al., 2013; Leri et al., 2005); however, extensive damage cannot be completely reversed, indicating that the regenerative potential of these stem cells during a damage challenge is limited (Chan et al., 2009; Hatzistergos et al., 2010B; Bailey et al., 2009). For the physiological cardiac hypertrophy observed in exercise training (Waring et al., 2014; Xiao et al., 2014b), pregnancy (Xiao et al., 2014a) or postnatal growth (Mann and Rosenzweig, 2012), little is known about the role of resident CSCs. Waring et al. (2014) have demonstrated that c-Kit$^+$ CSCs play an active role during physiological cardiac hypertrophy in mice specifically for exercise training. Whether the CMSCs are involved is not yet known. Because of the evidence supporting the existence of circulating stem cells capable of differentiating into cardiogenic cells (Beltrami et al., 2003; Quaini et al., 2002), it would also be worthwhile to investigate whether physical exercise training creates an attractive stimulus for noncardiac undifferentiated cells.

Therefore, the aim of the present study was to quantify the number of three different types of resident multipotent CSCs (c-Kit$^+$Lin$^-$, Sca-1$^+$Lin$^-$ and CMSCs) in a mouse model of physiologically hypertrophied hearts. Another aim was to determine whether extracardiac stem cells are recruited to the heart during physiological cardiac hypertrophy using a parabiotic eGFP transgenic/wild-type mouse model.

Methods

Experimental animals and groups

Male C57BL/6 mice and eGFP (enhanced Green Fluorescent Protein) transgenic mice, which constitutively express enhanced green fluorescent protein, were kept under controlled and stable conditions (temperature 22 °C, humidity 40–70% and light–dark cycle of 12/12 h) with free access to water and feed. All animals were obtained from the animal facilities of the Department of Physiology, Federal University of Triangulo Mineiro (UFTM) in Uberaba/MG, Brazil and received humane care in accordance with the ethical principles of animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA), and the protocol was approved by the UFTM Ethics Committee on Animal Use (Protocol number: 330).

The studied animals were allocated into four experimental groups: (i) Sedentary group; (ii) Trained group; (iii) Parabiotic Sedentary group; and (iv) Parabiotic Trained group.

Parabiosis

An eGFP transgenic male mouse at six weeks of age was surgically joined through the technique of parabiosis to a *wild-type* (WT) male mouse of the same age after at least a week in common housing (Bailey et al., 2006). The animals were anesthetized for complete muscular relaxation using ketamine and xylazine (100 mg/kg ketamine and 10 mg/kg xylazine) (Bailey et al., 2006). After the trichotomy of the corresponding lateral segments, an incision was made along the side of the body of the animals (from the olecranon to the knee joints) with dissection of the subcutaneous fascia to generate 0.5 cm of free skin (Wright et al., 2001). The knee and olecranon were united with 4.0 silk, and the skin was joined using a continuous suture with mononylon 6.0. Postoperative analgesia was provided using a 1.0 mg/kg i.m. dose of meloxicam, and the animals received autoclaved water supplemented with 0.16 mg/mL Baytril (McKinney-Freeman and Goodell, 2004) for seven days.